Attorney's Docket No.: 56446-20011.21/-017006 /D1240-7US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Jay SHORT et al.
Serial No. : 09/905,173
Filed : July 12, 2001
Title : ENZYMES HAVING TRANSAMINASE AND AMINOTRANSFERASE ACTIVITY AND METHODS OF USE THEREOF

Art Unit : 1652
Examiner : Elizabeth Slobodyansky, Ph.D.

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.132

Sir:

1. I, Jay M. Short, am a co-inventor with Patrick V. Warren, Ronald V. Swanson and Eric J. Mathur, on the above-identified patent application.

2. I am an expert in the field of molecular biology and enzyme development and was an expert at the time of the invention. I am presently employed as C.E.O. and as a research scientist at Diversa Corporation, San Diego, CA, assignee of the above-referenced patent application. My resume as documentation of my credentials is attached as Exhbit B.

3. I declare that at the time of the invention, aligning nucleic acid or polypeptide sequences was a routine method for comparing sequences to identify common structural characteristics (e.g., sequences, motifs) related to a function such as aminotransferase activity. One skilled in the art could have aligned and compared the disclosed exemplary sequences of the invention to each other or to known transaminase sequences to determine common, specific structural characteristics reasonably related to the genus of transaminases used in the claimed methods, e.g., as illustrated in Exhibit A. Exhibit A shows a sequence alignment among SEQ ID NOs 23 and 31, relevant to the claims in this application, and several other aminotransferases disclosed in this application.

34AT2_001 SEQ ID NOs: 23, 31 (relevant to the claims in this application)

3AT2_001 SEQ ID NOs: 35, 36

34AT5_001 SEQ ID NOs: 18, 26

34AT6_001 SEQ ID NOs: 39, 40

3AT1_001 SEQ ID NOs: 21, 29

(consensus sequence)

4. I declare that assays such as high through-put enzyme activity screening known at the time of the invention made methods obsolete and unnecessary that required previous knowledge of specific structural characteristics, e.g., protein structure, including secondary or tertiary structure, active site sequences, and the like. Assays such as high through-put enzyme activity screening known at the time of the invention made methods that required previous knowledge of how structure correlates with function, obsolete and unnecessary to practice the claim invention.

5. I declare that procedures for identifying nucleic acids that encode transaminase were conventional and routine in the art at the time of the invention. Procedures for identifying polypeptides having any transaminase activity (including enzymes capable of catalyzing the transfer of amino groups from α-amino to α-keto acids) were conventional and routine in the art at the time of the invention. Transaminase screening assays were routine and well known in the art at the time of the invention. Because the different reactions catalyzed by transaminases (aminotransferases), and assays for detecting such activity, were well known in the art at the time of the invention, one of ordinary skill in the art would have been able to ascertain the scope of the genus of transaminase-encoding nucleic acids used in the claimed methods with reasonable clarity and recognized that Applicants were in possession of the claimed invention at the time of filing.

6. I declare that one of ordinary skill in the art, using the teaching of the specification, could have made and expressed nucleic acids having a percent sequence identity

(including 70% sequence identity) to an exemplary nucleic acid, and could have determined using routine screening and with predicable positive results, which of those nucleic acids encoded a transaminase. Using the teaching of the specification one of ordinary skill in the art would have been able to ascertain the scope of the claimed genus of transaminase-encoding nucleic acids with reasonable clarity and recognized that Applicants were in possession of the claimed invention at the time of filing.

8. I declare that declares that it would not have required any knowledge or guidance as to how structure is related to function to generate the genus of transaminase-encoding nucleic acids used in the claimed methods without undue experimentation. Assays such as high through-put enzyme activity screening known at the time of the invention made methods that required previous knowledge of protein structure, including secondary or tertiary structure, active site sequences, and the like, obsolete and unnecessary. Assays such as high through-put enzyme activity screening known at the time of the invention made methods that required previous knowledge of how structure correlates with function obsolete and unnecessary to practice the claim invention. At the time of the invention, high through-put *in vivo* (e.g., whole cell) nucleic acid expression and enzyme activity screening protocols were well known in the art. Transaminase screening assays were well known in the art at the time of the invention. The specification presented to the skilled artisan a rational and predictable scheme for making the genus of transaminase-encoding nucleic acids used in the claim methods, including a rational and predictable scheme for modifying any nucleic acid residue of an exemplary nucleic acid with an expectation of obtaining the desired function. The specification provided sufficient guidance to one of ordinary skill in the art to make and use the claimed genus of nucleic acids or polypeptides to practice the invention.

9. I declare that one skilled in the art could have identified common structural characteristics distinguishing aminotransferases encoded by nucleic acids used in the claimed methods by simply aligning disclosed exemplary sequences of the invention to each other, as illustrated in Exhibit A, or to known transaminase sequences. The sequence alignment shown in Exhibit A illustrates that the exemplary sequence of the invention (SEQ ID NO:31) used in the

claimed methods has a plurality of shared sequence to other nucleic acids encoding polypeptides having transaminase activity. At the time of the invention aligning sequences was a routine method for comparing sequences to identify common structural characteristics (e.g., sequences, motifs) related to a function, for example, aminotransferase activity. One skilled in the art could have aligned and compared the disclosed exemplary sequences of the invention to each other or to known transaminase sequences to determine common, specific structural characteristics reasonably related to the genus of transaminases used in the claimed methods, e.g., as illustrated in Exhibit A.

10. I declare that procedures for modifying and expressing nucleic acids were conventional and routine in the art at the time of the invention. Procedures for determining the activity of the expressed modified nucleic acids and determining if the nucleic acids expressed a polypeptide with transaminase activity were conventional and routine in the art at the time of the invention. Procedures for determining sequence identity to an exemplary nucleic acid were routine in the art at the time of the invention. Procedures for expressing and screening for transaminase activity were conventional and routine in the art at the time of the invention. One of ordinary skill in the art using the teaching of the specification would have been able to make and use the genus of compositions used in the methods of the invention, including a genus of transaminase-encoding nucleic acids having at least 70% sequence identity to the exemplary nucleic acid without undue experimentation. It was considered routine by one skilled in the art at the time of the invention to screen for multiple substitutions or modifications of a nucleic acid or a polypeptide for functional variations, including screening for a genus of transaminase-encoding nucleic acids or a genus of transaminases. It was considered routine by one skilled in the art at the time of the invention to screen for subsequences of nucleic acids that can identify or encode enzymes (e.g., transaminases) or enzymatically active fragments of transaminases. It was considered routine by one skilled in the art at the time of the invention to screen for subsequences of nucleic acids that can identify by hybridization a polypeptide-encoding (e.g., transaminase-encoding) nucleic acid.

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted

Date: August 12, 2004

Jay M. Short